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Evaluation of Intestinal Clearance and Faecal Excretion of α_1-Antiproteinase and Immunoglobulins During *Crohn's* Disease and Ulcerative Colitis

By *Nathalie Kapel, Dominique Meillet, L. Favennec, Denis Magne, Denis Raichvarg* and *J.-G. Gobert*

Laboratoire de Coprologie — Groupe hospitalier Bichat-Claude Bernard — Paris

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Summary: The intestinal clearance of α_1-antiproteinase, monomeric IgA and IgG, and the daily fecal output of polymeric IgA and IgM were investigated in patients with inflammatory bowel diseases (inactive and active *Crohn*'s disease, ulcerative colitis) and in a control group. The intestinal clearance of α_1-antiproteinase was significantly increased in all patients with inflammatory bowel diseases ($p < 0.01$), irrespective of the grade of the disease. In contrast, increases in intestinal clearances of monomeric IgA and IgG were more closely related to the severity of the intestinal lesions. The associate determination of these three quantities should therefore be of interest for monitoring the degree of intestinal bowel inflammation. Faecal output of polymeric IgA was significantly increased in active intestinal disease ($p < 0.01$), whereas faecal IgM levels were not. The determination of the faecal output of polymeric IgA should contribute to the assessment of the activity of inflammatory bowel diseases, and may provide insight into the activation of the mucosal immune system.

Introduction

The intestinal clearance of α_1-antiproteinase ("α_1-antitrypsin") has been suggested as a marker of intestinal protein loss during inflammatory bowel diseases such as *Crohn*'s disease and ulcerative colitis (1, 2). However, the use of this quantity in the assessment of inflammatory bowel disease activity remains controversial (3, 4). Owing to the absence of a specific mucosal transfer system, the monomeric IgA of intestinal secretions appears to be derived mainly from passive transport of plasma monomeric IgA (5, 6). These monomeric IgA are relatively resistant to intestinal proteases and are found in the faeces in their native form or as fragments often bound to α_1-antiproteinase or pigments (7–9). Moreover, their intestinal clearance appears to be markedly increased during diarrhoea (10). *Jonard* et al. have demonstrated that IgG, present in the intestinal secretions of healthy subjects, are serum-derived (5). We therefore evaluated the value of determining monomeric IgA and IgG intestinal clearances, together with that of α_1-antiproteinase, for the purpose of exploring the degree of intestinal protein loss syndrome, which in turn is related to mucosal permeability. Moreover, it is well established that immunological events occur inside the inflamed *lamina propria*, causing changes in the relative proportions of Ig-producing cells (IgA, IgG and IgM) in the diseased mucosa, as well as in *in vitro* Ig secretion by mononuclear cells of the *lamina propria* (11–16). In healthy subjects, intestinal humoral immunity is mainly mediated by polymeric IgA, with 70 to 95 percent of secretory IgA partially protected from proteolysis by the extra-epithelial moiety of the poly-Ig receptor i.e. the secretory component (17–19). IgM, monomeric IgA and IgG play a less important role in this setting (5, 17, 18). The determination of the faecal output of polymeric IgA and IgM may provide a means of evaluating, *in vivo*, the repercussions of inflammatory bowel disease on local humoral immunity, which in turn represents an evaluation of the activity of the disease.

The present work was therefore undertaken to determine the intestinal clearance of α_1-antiproteinase,

monomeric IgA and IgG, as well as faecal output of polymeric IgA and IgM in an homogeneous population of patients with active and inactive *Crohn*'s disease and ulcerative colitis, in comparison with normal controls. The diagnostic and prognostic values of these functions in monitoring intestinal protein loss, and characterizing the pathophysiology and immunologic abnormalities of the humoral intestinal system were evaluated.

Patients and Methods

Population

Four groups of patients were studied. The control group consisted of 20 healthy adults (10 females, median age 48 years, range 19–75; 10 males, median age 37 years, range 20–62) with no intestinal disturbances and normal faecal constituent values. Of 57 selected patients with inflammatory bowel disease, 25 had various degrees and (or) localisations of *Crohn*'s disease, the diagnosis dating from two to twenty-three years. They were separated into two groups on the basis of the *Crohn*'s disease activity index (20). The first consisted of 10 patients (3 females, median age 22 years, range 19–42; 7 males, median age 29 years, range 22–66) with inactive *Crohn*'s disease (activity index $\leq$ 150). Four of these patients had ileitis, 3 had ileocolitis, and 2 had pure colitis disease. The second group consisted of 15 patients (9 females, median age 33 years, range 21–67; 6 males, median age 41 years, range 21–59) with active *Crohn*'s disease (activity index > 150). Of these patients, 4 had ileitis, 5 had ileocolitis, and 6 had pure colitis disease. The remaining 32 patients (20 females, median age 39 years, range 12–72; 12 males, median age 44 years, range 17–53) had ulcerative colitis, diagnosed six months to twenty-one years previously.

Preparation of samples

Twenty-four-hour stools were collected on three consecutive days and weighed. Occult blood was sought using routine guaiac tests (Hemocult II®, Smithkline diagnostics, USA and Hemofec®, Boehringer Mannheim, Germany). After mixing the 3-days sample from each subject with sodium azide (1 g/l final concentration) and di-isopropylfluorophosphate (Sigma, Saint Louis MI, USA) at a final concentration of 1 mmol/l, five grams of the homogenized material was removed and vigorously stirred for 1 h with 10 ml of 0.15 mol/l NaCl at + 4 °C. After centrifugation (10 000 *g*, 10 min), the supernatant was immediately frozen to −20 °C. All assays were performed using these three-fold-diluted stool samples. Five ml of blood was drawn from each subject on the last day of stool collection and the serum was stored at −20 °C until use.

α_1-Antiproteinase determination

Serum and faecal α_1-antiproteinase were measured by single radial immunodiffusion. Nor-Partigen® plates (Behring-diagnostic, Rueil-Malmaison, France) were used for undiluted serum while LC-Partigen® plates (Behring) were used for 1 : 9 and 1 : 30 diluted faecal samples to avoid zone phenomena.

Measurement of total IgA, monomeric IgA and polymeric IgA

Total IgA and monomeric IgA were measured in serum and faeces by means of a modified electroimmunodiffusion method according to *Meillet* et al. (10). The electroimmunodiffusion was run for 18 h at + 4 °C (2 V cm^{-1}) in an agarose-polyacrylamide gel, in the presence of a gel barrier which blocked the migration of proteins with relative molecular masses M_r > 200 000. Two series of deposits, with 5 µl of samples or standards [purified monomeric IgA, 14.3 g/l, prepared according to the method of *Fine* (21)], were made on both sides of the blocking gel. Samples placed above the blocking gel gave the total IgA by comparison with the standard. A correction factor of 1.8 was applied to take into account the precipitation peak height difference between monomeric IgA and polymeric IgA for the same concentration. The monomeric IgA concentration was determined, without correction, by placing the samples and the standards under the blocking gel and comparing the results with the standard curve. The difference between the two values represented polymeric IgA.

Measurement of IgG and IgM

IgG and IgM were measured in an electroimmunodiffusion assay using 1% IEF-agarose (Isogel®, Pharmacia, Uppsala, Sweden) containing 0.5 percent antiserum (anti-γ and anti-µ, respectively; Dakopatts, Denmark). Serum samples diluted 1 : 20 and undiluted faecal extracts were measured against serum standards (Protein standard serum LC-A and LC-V, Behring). The assays were run for 18 h at + 4 °C at a potential gradient of 2 V cm^{-1} in a migration buffer composed of 0.03 mol/l, pH 8.4 Tris-barbital.

Presentation of results

Daily faecal outputs of IgM and polymeric IgA were expressed as mg/24 h. Intestinal clearances of α_1-antiproteinase, monomeric IgA and IgG were calculated according to the usual formula and expressed as ml/24 h (2).

Statistical analysis

The specificity and the sensitivity of the biological markers were calculated. The upper limit of normal for faecal constituent values was defined as the mean of control values plus two standard deviations. Since the data did not conform to a *Gaussian* distribution, the results are given as median and range, and were compared using the non-parametric *Mann & Whitney* "U" test. Correlations were studied by means of *Spearman*'s correlation test.

Results

The median fresh weight of faeces was significantly increased ($p < 0.01$) in patients with inflammatory bowel disease: 120 g/24 h (range 65–255) for controls, 197 g/24 h (range 47–379) for patients with inactive *Crohn*'s disease, 306 g/24 h (range 119–699) for patients with active *Crohn*'s disease and 210 g/24 h (range 59–966) for patients with ulcerative colitis. Faecal occult blood was detected in none of the patients with quiescent *Crohn*'s disease, in 5 patients with active *Crohn*'s disease and in 17 patients with ulcerative colitis. Intestinal clearances of α_1-antiproteinase, monomeric IgA and IgG in patients and normal controls are illustrated in figure 1. Relationships between *Crohn*'s disease activity index and these clearances are presented in figure 2. α_1-Antiproteinase clearance was significantly raised ($p < 0.01$) in all the patients with inflammatory bowel disease, compared

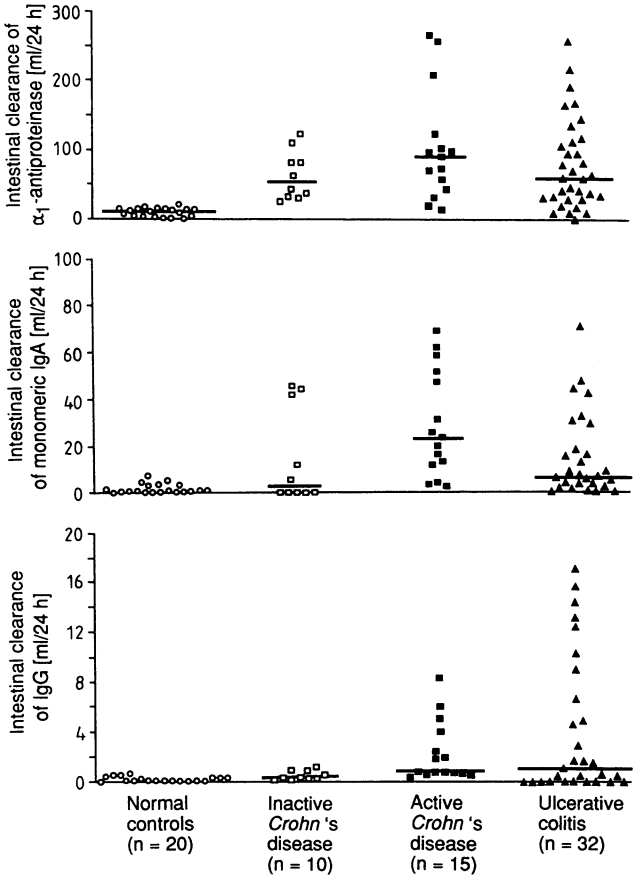

Fig. 1. Intestinal clearance of α_1-antiproteinase, monomeric IgA and IgG (ml/24 h) in normal controls, patients with inactive and active *Crohn*'s disease, and patients with ulcerative colitis. The medians are indicated by a horizontal line.

with the normal controls. The median values obtained in each group were 10.5 ml/24 h (range 0.4–16) for controls, 51.6 ml/24 h (range 25.3–122.2) for patients with inactive *Crohn*'s disease, 91.2 ml/24 h (range 12–265.6) for patients with active *Crohn*'s disease and 59.8 ml/24 h (range 0–258.8) for those with ulcerative colitis. Using an upper limit of normal of 20 ml/24 h, this value increased with perfect specificity (100%) and good sensitivity (inactive *Crohn*'s disease: 90%; active *Crohn*'s disease: 93%; ulcerative colitis: 81%). However, the increase was not statistically correlated with the grade of the disease. The median intestinal clearance of monomeric IgA was 0.8 ml/24 h (range 0–7.6) for controls, 1.8 ml/24 h (range 0–45.7) for patients with inactive *Crohn*'s disease, 23.3 ml/24 h (range 2.3–68.9) for patients with active *Crohn*'s disease and 6.9 ml/24 h (range 0–70.9) for those with ulcerative colitis. The upper limit of normal was fixed at 7 ml/24 h. Under these conditions, intestinal clearance of monomeric IgA increased with good specificity (95%), similar to that of α_1-antiproteinase, whereas its sensitivity appeared to be related to the disease activity: 73% of patients with active *Crohn*'s disease and 53% of those with ulcerative colitis had an elevated intestinal clearance of monomeric IgA against only 40% of those with inactive *Crohn*'s disease. Statistical analysis showed a significant increase ($p < 0.01$) in intestinal clearance of monomeric IgA only in patients with active *Crohn*'s disease and ulcerative colitis. This value appeared to be well cor-

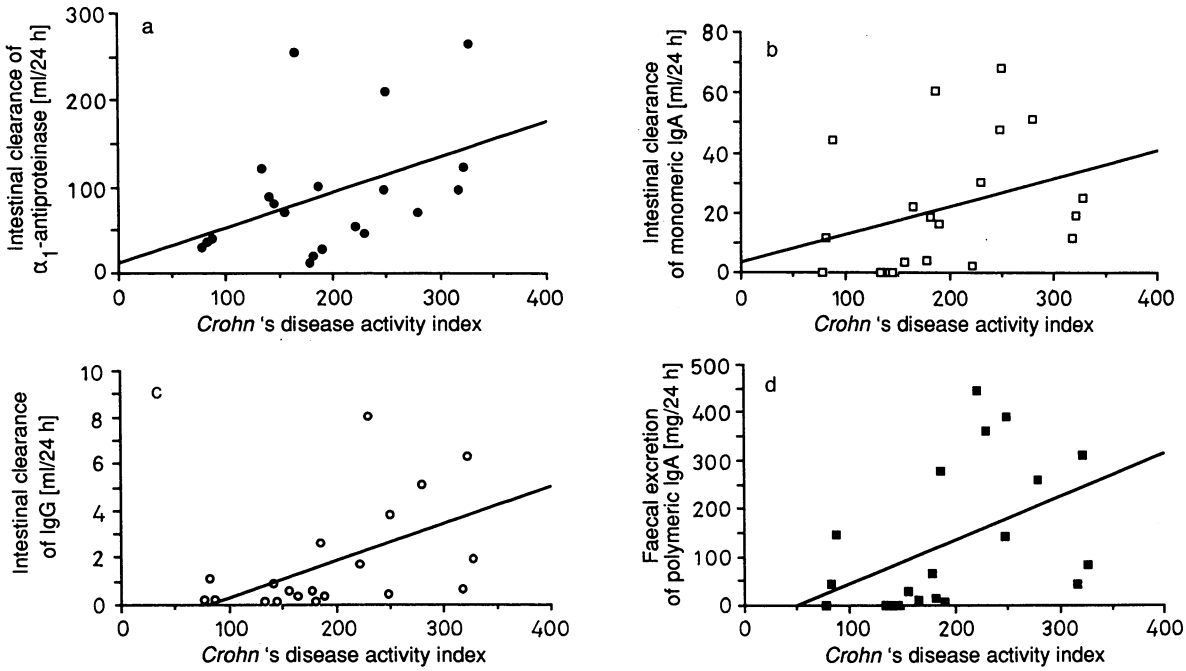

Fig. 2. Relationship between *Crohn*'s disease activity index and
(a) intestinal clearance of α_1-antiproteinase ($r = 0.435$, $p < 0.1$);
(b) intestinal clearance of monomeric IgA ($r = 0.539$, $p < 0.02$);
(c) intestinal clearance of IgG ($r = 0.620$, $p < 0.01$) and
(d) daily faecal excretion of polymeric IgA ($r = 0.605$, $p < 0.01$).

related to the grade of the disease in *Crohn*'s disease. No correlation between α_1-antiproteinase clearance and monomeric IgA clearance was found, except for patients with active *Crohn*'s disease ($p < 0.05$). The median intestinal clearance of IgG in the control group was 0.13 ml/24 h (range 0.03 – 0.52). This value was significantly increased in patients with inactive *Crohn*'s disease ($p < 0.05$) (median value 0.26 ml/24 h, range 0.07 – 1.16), in patients with active *Crohn*'s disease ($p < 0.01$) (median value 0.7 ml/24 h, range 0.15 – 8.2) and in those with ulcerative colitis ($p < 0.01$) (median value 1.0 ml/24 h, range 0.02 – 17.1). The upper limit of normal of this value was fixed at 0.5 ml/24 h. It increased with good specificity (92%) and sensitivity, similar to that of monomeric IgA intestinal clearance: 30% of patients with inactive *Crohn*'s disease, 73% of those with active *Crohn*'s disease and 56% of those with ulcerative colitis showed elevated intestinal clearance of IgG. Regardless of the type and grade of inflammatory bowel disease, no correlation was found between the intestinal clearances of α_1-antiproteinase and IgG. In contrast, intestinal clearances of monomeric IgA and IgG correlated well in patients with active *Crohn*'s disease ($p < 0.05$) or ulcerative colitis ($p < 0.01$).

The faecal output of IgM was not affected by inflammatory bowel disease: the median values obtained in each group were 1.4 mg/24 h (range 0.5 – 2.6) for controls, 1.9 mg/24 h (range 1 – 5.7) for patients with inactive *Crohn*'s disease, 3.5 mg/24 h (range 1.0 – 18.0) for patients with active *Crohn*'s disease and 3.0 mg/24 h (range 1.0 – 36.2) for those with ulcerative colitis. For polymeric IgA, the median faecal output in each group was 2.0 mg/24 h (range 0.8 – 81.8) for controls, 10.5 mg/24 h (range 2.5 – 293.5) for patients with inactive *Crohn*'s disease, 141.3 mg/24 h (range 8.9 – 443.9) for patients with active *Crohn*'s disease and 135.5 mg/24 h (range 4.5 – 686.8) for those with ulcerative colitis (fig. 3). Statistical analysis showed a significant increase in the faecal output of polymeric IgA in patients with quiescent *Crohn*'s disease ($p < 0.05$), active *Crohn*'s disease ($p < 0.01$) and ulcerative colitis ($p < 0.01$). However, this increase was found in only 33% of the patients with inactive *Crohn*'s disease, compared with 67% and 78% of those with active *Crohn*'s disease and ulcerative colitis, respectively, and it appeared to be correlated to the grade of the disease (fig. 2). Moreover, an increase of 15% in the monomeric IgA/total ratio was found in patients with inflammatory bowel disease, relative to the control group.

None of the studied laboratory quantities correlated with the exact location of the disease.

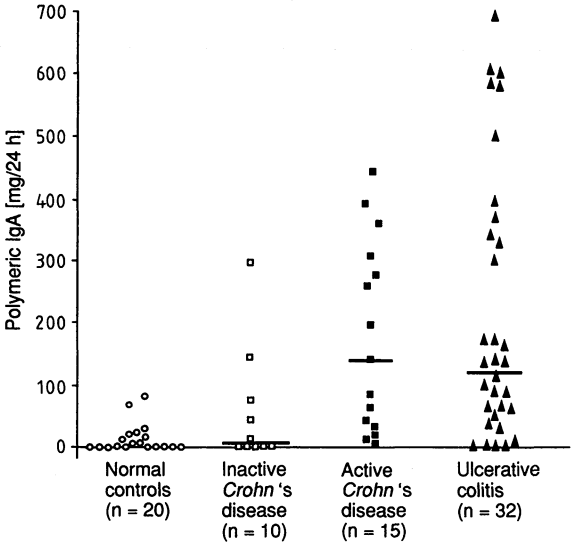

Fig. 3. Daily faecal excretion of polymeric IgA (mg/24 h) in normal controls, patients with inactive and active *Crohn*'s disease, and patients with ulcerative colitis. The medians are indicated by a horizontal line.

Discussion

Our findings confirm the value of the intestinal clearance of α_1-antiproteinase for screening for intestinal protein loss syndrome with good specificity and and sensitivity (22). However, this quantity alone appears to be insufficient for monitoring the course of inflammatory bowel disease, since no statistical difference in the intestinal clearance of α_1-antiproteinase was found between patients with active and inactive *Crohn*'s disease. These data are in agreement with those of *Mizon* et al. who demonstrated that α_1-antiproteinase was excreted in faeces in three different molecular forms. One deglycosylated component of M_r 38 000, which can be underestimated in radial immunodiffusion, is recovered in normal faecal extract and in some patients with inactive *Crohn*'s disease, while two other components of M_r 45 000 and M_r 51 000 respectively, are found in patients with active *Crohn*'s disease (23).

Although *O'Mahony* et al. detected only very small amounts of immunoglobulins in semi-liquid faecal specimens from gut lavage (24), we found a significant increase in the intestinal clearance of monomeric IgA and IgG in most of our patients with active inflammatory bowel disease, correlated with the grade of disease activity. This discrepancy was related to the non-iatrogenic nature of our specimens and to the relative resistance of monomeric IgA to intestinal proteases. The protease susceptibility and faecal occurrence of IgG are poorly documented, but these aspects are now under investigation in our laboratory.

Since far greater increases in intestinal clearances of monomeric IgA and IgG were found in most of the patients with active *Crohn*'s disease than in those with inactive *Crohn*'s disease, these quantities appear to reflect increased intestinal permeability. It therefore seemed to be of interest to determine both the intestinal clearance of monomeric IgA and IgG and that of α_1-antiproteinase, in order to monitor the degree of intestinal bowel inflammation.

Moreover, this study supports previous immunohistochemical findings showing that the number of IgA-producing cells per mucosal tissue unit (gut lengh unit) is increased according to the degree of inflammation (12). In spite of the described reduction in J chain expression during inflammatory bowel disease, polymeric IgA production is sufficiently maintained to afford an enhanced response of secretory IgA (25). This increase in faecal polymeric IgA output, especially in patients with active *Crohn*'s disease or ulcerative colitis, confirmed that the activation of the mucosal lymphoid system during inflammatory bowel disease was proportional to the grade of the disease. In addition, our results provide information on the *in vivo* activation of the mucosal immune system during inflammatory bowel disease. Although the pathogenic mechanisms or antigens leading to these abnormalities remain to be identified, the determination of the daily faecal output of polymeric IgA may be of value for assessing intestinal immunity during inflammatory bowel disease, and the grade of the disease.

Conclusion

Our study, performed on an homogeneous population of patients with inflammatory bowel disease assesses the value of studying faecal constituents. It reveals large differences between controls and inflammatory bowel disease patients in terms of intestinal clearance of α_1-antiproteinase, IgG and monomeric IgA, as well as faecal polymeric IgA output. However, because of the large overlap between the results obtained in active and inactive *Crohn*'s disease, particulary for the two Ig clearances, none fo the studied constituents can be considered as a marker of inflammatory bowel disease activity. In contrast, the combined study of the intestinal clearance of α_1-antiproteinase, monomeric IgA and IgG, together with daily faecal output of polymeric IgA may provide a useful non-invasive tool for assessing inflammatory bowel disease activity and monitoring therapeutic efficacy.

To confirm the value of these measurements during intestinal disease, we are now extending our investigations to other kinds of inflammatory or exsudative bowel disease, especially in HIV patients with and without opportunistic digestive infections.

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Dr. Nathalie Kapel
Laboratoire de Coprologie
7 rue du Fer à Moulin
F-75005 Paris